American Journal of Hematology 53:196–200 (1996)

Cytophilic Immunoglobulin G Binding on Neutrophils From a Child With Malignant Osteopetrosis Who Developed Fatal Acute Respiratory Distress Mimicking Transfusion-Related Acute Lung Injury

Prema R. Madyastha, Elaine K. Jeter, and L. Lyndon Key, Jr.

Section of Transfusion Medicine, Department of Pathology and Laboratory Medicine/Pediatrics (P.R.M., E.K.J.)., and Division of Endocrinology, Department of Pediatrics (L.L.K.), Medical University of South Carolina, Charleston, South Carolina

A 16-month-old boy, diagnosed at age 3 months with osteopetrosis, was treated since age 6 months with rhIFN-γ in combination with rhM-CSF. The child developed acute respiratory distress within 1 hr of a paternal platelet transfusion. Both the child and the father were blood group type O, and platelets were collected the previous day from the father. Chest X-ray revealed right pulmonary consolidation and a complete "whiteout" on the left. By 24 hr, the lungs had the appearance of adult respiratory distress syndrome (ARDS). Over the course of the next 11 days, the child remained intubated and hypotensive, and died of respiratory insufficiency 11 days later. ARDS was confirmed at autopsy. Pre- and posttransfusion patient's sera, as well as paternal serum, were tested by granulocyte agglutination and flow cytometry against granulocytes (PMN) from the patient, father, mother, and routine cell-panel donors and lymphocytes for the presence of neutrophil-specific and lymphocyte (HLA) antibodies, to rule out classical transfusion-related acute lung injury (TRALI). Both the patient's and the paternal sera were devoid of antibodies, but the patient's neutrophils demonstrated strong binding of cytophilic IgG accompanied by extremely low serum IgG and IgG1 levels. Since rhIFN-γ is known to upregulate Fc gamma receptor type I (FcγRI) with high affinity for IgG1, the binding of cytophilic IgG suggests that the patient's neutrophils may have been activated in vivo. The case report of another child with osteopetrosis has also been described. Although the blood specimen was not available for serological studies, this 4½-year-old child treated with rhIFN-γ and rhM-CSF also died of adult respiratory distress syndrome, with similar clinical presentations. © 1996 Wiley-Liss, Inc.

Key words: platelets, transfusion-related acute lung injury, recombinant human interferon gamma, recombinant human macrophage colony-stimulating factor, polymorphonuclear leukocytes, neutrophils, Fc gamma receptor type I

INTRODUCTION

Osteopetrosis is a heterogenous group of bone disorders associated with deficiencies in osteoclastic bone resorption and leukocyte functions [1]. Patients with osteopetrosis have been treated with recombinant human interferon gamma (rhIFN-γ) and/or macrophage colony-stimulating factor (rhM-CSF), resulting in improvement in bone resorption and immune functions [2,3]. We describe the case of a child with malignant osteopetrosis treated with rhIFN-γ and rhM-CSF who developed a fatal acute lung injury following transfusion of platelets from his father.

Received for publication December 11, 1995; accepted July 10, 1996.

This paper was presented in part at the Third European Symposium on Platelet and Granulocyte Immunobiology, Robinson College, Cambridge, UK, June 1994, and at the 47th Annual Meeting of the American Association of Blood Banks, San Diego, CA, November 1994.

Address reprint requests to Prema Madyastha, Ph.D., Department of Pediatrics/Pathology and Laboratory Medicine, Rm. 316, Clinical Science Building, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

CASE REPORTS

Patient 1

A 16-month-old boy, diagnosed at age 3 months with osteopetrosis, was treated since age 6 months with rhIFN-γ in combination with rhM-CSF. A bone biopsy was performed under general anesthesia. A preoperative chest X-ray was negative for pulmonary disease, and the laboratory values were: hemoglobin, 8.7, and platelet count, 25,000. Immediately prior to the procedure, the child received a 100-ml aliquot of apheresis platelets that had been collected the previous day from the child's father. The child and the father were blood group ABO compatible. A second 100-ml aliquot was adminstered immediately following the bone biopsy. One hr later, the patient experienced a sudden increase in respiratory rate, a drop in blood pressure, and decreased oxygen saturation prior to respiratory arrest. A repeat chest X-ray revealed right pulmonary consolidation and a complete "whiteout" on the left. There was no evidence of aspiration. No organisms were cultured from bronchial aspirates. By 24 hr, the lungs had the appearance of adult respiratory distress syndrome (ARDS), with a ground-glass appearance on both sides with no evidence of lobar consolidation. Over the course of the next 11 days, the child remained intubated and hypotensive, and died of respiratory insufficiency 11 days later. ARDS was confirmed at autopsy, with hyaline membranes present in all sections. There was no evidence of pulmonary embolus or bleeding, except for damage from high-pressure ventilation. The total serum IgG (293 mg/dl) and IgG1 (1.98 g/l) levels were extremely low in this patient as compared to reference range values for age-matched controls (IgG, 345–1,213 mg/dl; IgG1, 2.86–6.80 g/l).

Patient 2

A 4½-year-old boy from Brazil who was diagnosed with malignant osteopetrosis at age 15 months was treated with low-dose prednisone and received monthly RBC and platelet transfusions prior to coming to the United States. At age 3 years, clinical features included blindness, severe developmental delays, recurrent infections, severe thrombocytopenia, and pathologic fractures. At age 4 years the child's spleen extended to the pelvic brim, and the liver was 6 cm below the R costal margin. hemoglobin was 7.1 gm/dl, with a platelet count of 31,000. The family returned to the U.S. to begin rhIFN-γ/M-CSF therapy. After 3 weeks of therapy, M-CSF was stopped due to severe thrombocytopenia. Interferon was continued. During the next 6 months, the child continued to require RBC/platelet transfusions with increasing frequency, and antibiotics for recurrent infections. He was admitted at age 4½ years for his 6-month evaluation and discharged. He was readmitted 3 days later for elective myringotomy with tube placement under general anesthesia. Intraoperatively a single unit of leukocyte-depleted, irradiated platelets and RBCs were administered. In recovery, he was extubated without difficulty. Approximately 1 hr later, he suddenly became tachypneic, bradycardic, and hypotensive. An X-ray showed bilateral "ground-glass" appearance, and despite fluid and ionotropic support, the child died approximately 36 hr after surgery. An autopsy was performed. Severe pronounced hyaline membrane formation, characteristic of adult respiratory distress sndrome, was noted throughout all lung fields. There was no evidence of aspiration or pulmonary hemorrhage. Blood samples from this patient were not available for serological investigations.

MATERIALS AND METHODS

Blood Samples

Blood samples from the father of patient 1 and from patient 1 (pre- and posttransfusion) were obtained for serological investigations to rule out a reaction that appeared to represent transfusion-related acute lung injury (TRALI). Blood samples obtained from the mother and from routine cell-panel donors were used as controls. The study was approved by the Institutional Review Board for Human Research, Medical University of South Carolina, and informed consents were obtained from participants prior to entry into the study.

Screening of Sera for Anti-Neutrophil and Anti-Lymphocyte (HLA) Antibodies

Sera from the father and the patient were tested against granulocytes (PMN) isolated [4] from the father, patient, and controls. Sera were also tested by the routine cell-panel assay against PMN from several normal donors [5]. The conventional granulocyte agglutination technique [5] and a granulocyte flow cytometry technique [6] were employed to detect antibodies. HLA antibodies were screened by the tissue-typing laboratory utilizing standard techniques.

Granulocyte Flow Cytometry Technique

The sera (10 μl) were first incubated with equal volumes of PMN suspension (25×10^6/ml) for 15 min at room temperature. After washing three times, 50 μl of fluorescein-conjugated goat $F(ab')_2$ antihuman IgG (Organon Teknika Corp., West Chester, PA) were added in a predetermined dilution and incubated for 30 min at room temperature. After washing, the percent fluorescent positive cells (% FPC) were determined using an EPICS II flow cytometer (Coulter Electronics, Hialeah, FL). The control cells were electronically gated to determine the cytogram of PMN, and 5,000 events were analyzed. The histogram (green fluorescence) and % FPC were obtained

to determine the background reading due to cells alone, which was set at <5. Further analyses were performed utilizing this setting. Serum from a nontransfused blood group AB male donor was used as the negative (NC-Se) control. Serum obtained by pooling several sera from patients with multiple transfusions containing strong PMN antibodies was used as a positive (PC-Se) control. Serum that reacted with ≤13% FPC was considered negative for neutrophil antibody [6].

RESULTS

Absence of Anti-Neutrophil Antibody in Sera From the Patient and the Father and Detection of Cytophilic IgG on the Patient's PMN

Both pre- and posttransfusion patient's sera, as well as paternal sera, failed to react with PMN from the father, mother, and routine cell-panel donors, suggesting the absence of PMN antibody (Table I). However, the patient's PMN were nonspecifically agglutinated in the presence of control or test sera. This pattern of reactivity was also detected by granulocyte flow cytometry technique. The histograms demonstrating these patterns are illustrated in Figure 1. These histograms show the % FPC with PMN from the father (Fig. 1a), and the patient (Fig. 1b) obtained when tested with control or test sera. The pattern of histograms with maternal PMN was identical to that of paternal histograms (Fig. 1a) and therefore was not included in Figure 1. The first four histograms in each row (Fig. 1a,b) represent the % FPC obtained with controls. These are: 1) Buff, PMN treated with buffer only without fluorescent reagent to obtain minimum fluorescence due to cells alone (2% FPC); 2) FITC-IgG, PMN first treated with buffer without serum and then stained with FITC-IgG to obtain background fluorescence due to FITC-IgG (Fig. 1a, 4%; Fig. 1b, 92%); 3) NC-Se, PMN treated with negative control serum and FITC-IgG (Fig. 1a, 7%; Fig. 1b, 87%); and 4) PC-Se, PMN treated with positive control serum and FITC-IgG (Fig. 1a, 95%; Fig. 1b, 97%). These data indicated that, as expected, parental PMN (Fig. 1a, control cells) did not have any IgG inherently bound to the cell surface, whereas the high % FPC obtained with patient PMN (Fig. 1b) in the presence of buffer without serum (FITC-IgG) or NC-Se revealed the presence of cell-bound IgG. The histograms from rows 5–7 represent % FPC obtained with test sera. These are: 5) Pt-Pre, PMN treated with patient pretransfusion serum (Fig. 1a, 4%; Fig. 1b, 93%); 6) Pt-Post, PMN treated with patient posttransfusion serum (Fig. 1a, 5%; Fig. 1b, 95%); and 7) Fa, PMN treated with paternal serum (Fig. 1a, 4%; Fig. 1b, 89%). The low % FPC obtained when patient sera were treated with parental PMN (Fig. 1a) further indicated the absence of neutrophil antibody. However, the high % FPC obtained with patient PMN (Fig. 1b) reflected the inherently bound IgG, independent of the binding of IgG from control or test sera used in the assay. Testing of the paternal serum in the cell-panel assay also confirmed the absence of neutrophil antibodies. HLA antibodies were absent in patient and parental sera. These results indicate nonspecific adherence of cytophilic IgG on patient's PMN.

TABLE I. Screening of Patient 1 and Parental Sera for Anti-Neutrophil Antibody

	Granulocyte agglutination[a]			
	PMN[b]			
Sera	Fa	Mo	Pt	Do
Control sera[c]				
Mo	0	0	+	0
Do	0	0	+	0
Nc-Se	0	0	+	0
PC-Se	+	+	+	+
Test sera[d]				
Pt-pre	0	0	+	0
Pt-post	0	0	+	0
Fa	0	0	+	0

[a]Granulocyte agglutination was arbitrarily graded as 0, negative agglutination, and +, strong agglutination.
[b]Test cells: Fa, father; Mo, mother; Pt, patient; Do, donor (a representative cell-panel donor).
[c]Control sera: Mo, mother; Do, donor; NC-Se, negative control (serum from a nontransfused blood group AB male donor); PC-Se, positive control (serum obtained by pooling several sera from recipients of multiple transfusions containing neutrophil antibody).
[d]Test sera: Pt-pre, patient pretranfused; Pt-post, patient posttransfused; Fa, father.

DISCUSSION

In the present report, we describe the first known cases in 2 young children (patient 1, age 16 months, and patient 2, age $4\frac{1}{2}$ years) with malignant osteopetrosis, of a fatal respiratory distress syndrome that mimicked TRALI after transfusion of paternal or allogeneic pheresis platelets. Both patients were on treatment with rhIFN-γ and rhM-CSF for osteopetrosis. Detailed serological investigations were performed in one patient (patient 1); however, blood samples from patient 2 were not available for study. In patient 1, neutrophil-specific and HLA antibodies were absent in both the patient's and the paternal sera. The patient's PMN, however, demonstrated strong binding of IgG on the cell surface. The absence of neutrophil-specific and HLA antibodies in the patient's and paternal sera indicated that the IgG detected on the patient's PMN was not due to the binding of these antibodies to surface antigens. The nonreactivity of the patient's sera with parental PMN further suggested that the surface-bound IgG was not due to binding of the neutrophil autoantibody [5]. These results strongly suggest that the cell-bound IgG is due to nonspecific binding of cytophilic IgG unrelated to specific antibody.

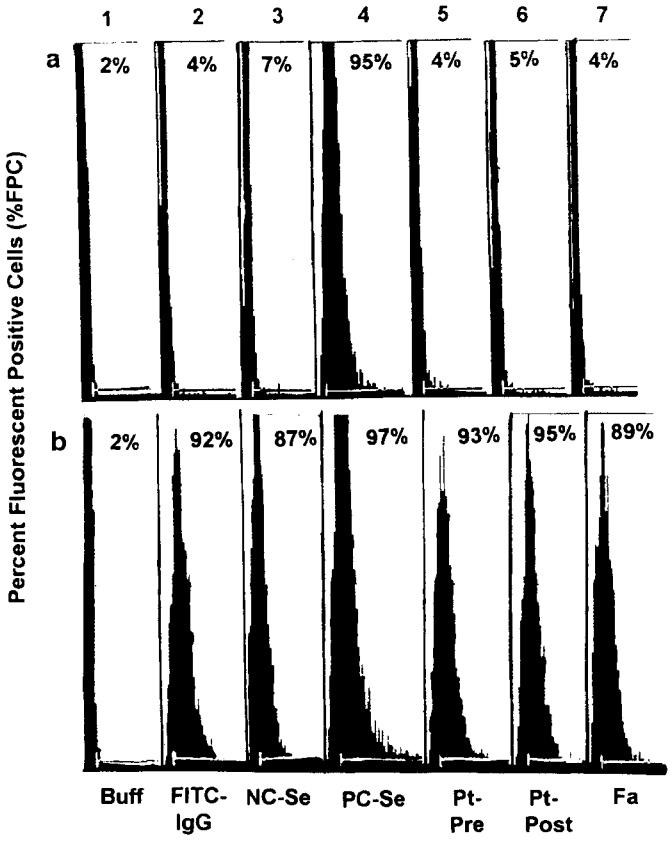

Fig. 1. Flow cytometry histograms of paternal and patient PMN tested with control, patient, and parental sera. These histograms show the % FPC with PMN from the father of patient (a) and from patient 1 (b), obtained when tested with control or test sera. The pattern of histograms with maternal PMN was identical with that of paternal histograms (a), and therefore was not included. The first four histograms in each row (a,b) represent % FPC obtained with controls. These are: (1) Buff, PMN treated with buffer only without fluorescent reagent to obtain minimum fluorescence due to cells alone; (2) FITC-IgG, PMN first treated with buffer without serum and then stained with FITC-IgG to obtain background fluorescence due to FITC-IgG; (3) NC-Se, PMN similarly treated with negative control serum; and (4) PC-Se, PMN treated with positive control serum. Histograms 5–7 represent % FPC obtained with test sera. These are: (5) Pt-Pre, (6) Pt-Post, and (7) Fa, PMN treated with patient pretransfusion, patient posttransfusion, and paternal serum, respectively. PMN treated with maternal or donor sera gave similar histograms and are not included. Controls as well as test sera were negative for neutrophil antibody (≤13% FPC) when tested against PMN from the father (a). In contrast, the patient's PMN (b), treated with buffer or test sera and then stained with FITC-IgG, showed strong binding of IgG (87–97% FPC), indicating presence of cytophilic IgG.

In patients who experience TRALI, typical clinical presentations include acute respiratory distress characterized by hypoxemia, hypotension, fever, and pulmonary edema with X-ray evidence of bilateral pulmonary infiltrates usually developing within 1–4 hr after transfusion [7]. In extremely severe cases, TRALI may resemble adult respiratory distress syndrome. The interactions of neutrophil-specific alloantibody present in the donor serum with antigens present on recipient neutrophils have been reported to cause TRALI [8]. Although the implications of cytophilic IgG in TRALI are unknown at this time, the absence of neutrophil-specific and HLA antibodies in the patient's and parent's sera clearly indicate that specific antibodies were not the causative agents for the TRALI-like episode (TLE) in this patient. The possibility that immune complexes containing anti-A or anti-B may have formed and bound to other FC receptors on neutrophils is ruled out, since the father and the child were ABO-compatible. Currently we have not looked for increased levels of neutrophil-associated IgG in patients with acute respiratory distress syndrome not related to transfusion, and it is not known whether during acute lung injury several other inflammatory factors that may be released stimulate neutrophils, causing them to bind IgG. It is also unlikely that cytokines or platelet-activating factor-like lipids that are known to be generated in blood products stored for 3 weeks or longer [9] stimulated the patient's neutrophils that resulted in the binding of IgG triggering the TRALI reaction, since the platelet product was collected the previous day. Our recent observations in several untreated patients [10] revealed PMNcp-IgG binding, which further suggests that binding of cytophilic IgG may not be a marker of TRALI reaction, but might have been involved in the etiology of the disease process.

Neutrophils from patients treated with rhIFN-γ or recombinant human granulocyte colony-stimulating factor (rhG-CSF) express high quantities of Fc gamma receptor type I (CD64 molecules), with high affininty for monomeric IgG (IgG1) [11,12]. It is not known whether these patients had received any blood products or had experienced transfusion reactions. Although we did not directly measure FcγRI expression, it is possible that PMN were activated in vivo by rhIFN-γ, which caused the upregulation of FcγRI with concurrent uptake of serum IgG1 on these receptors. This is supported by our data (manuscript submitted), obtained subsequently in several other patients treated with rhIFN-γ, that clearly indicate an upregulation of FcγRI, and that may have an additional relevance to the fact that the total serum IgG (293 mg/dl) and IgG1 (1.98 g/l) levels were extremely low in this patient as compared to the reference range values for age-matched controls (IgG, 345–1,213 mg/dl; IgG1, 2.86–6.80 g/l). We speculate that binding of monomeric IgG1 to FcγRI receptors not only reflects the activation of PMN, but also may induce surface-membrane changes that result in the triggering of specific responses. Other possible causes for this picture could be a fat embolus; however, in this patient with osteopetrosis, the bone-marrow biopsy specimen was devoid of fat.

The occurrence of ARDS in patients treated with GM-CSF or G-CSF has recently been reported [13,14]. The

increased expression by GM-CSF of the glycoproteins CD11b/CD18 on the surface of neutrophils, promoting adhesiveness of neutrophils to the pulmonary endothelium coupled with the release of superoxide anions by GM-CSF-primed neutrophils, has been implicated as a possible mechanism involved in the pathogenesis of ARDS. It is not clear whether the patients treated with G-CSF [13] received blood transfusions; however, the patient treated with GM-CSF had severe transfusion-dependent refractory anemia [13]. Although the role of transfusion in TLE as observed in the 2 patients in our study is currently unknown, caution is warranted, and physicians using GM-CSF, G-CSF, or either of those in combination with rhIFN-γ, should be aware of the potential for acute lung injury, especially if the patient is to receive concurrent blood-component therapy.

IFN-γ and the monocyte-derived cytokines have been shown to increase the expression of CD11b/CD18 or PMN and the expression of leukocyte-adhesion molecules on endothelial cells [15,16], which are important in the initial process of PMN mobilization to an inflammatory site. Although we were unable to measure the PMN expression of various cell-adhesive molecules in patient 1, the fact that IFN-γ does not directly stimulate PMN migration but can influence the mobilization and function of PMN through its synergistic activity with other cytokines [17] may suggest a similar mechanism in this patient.

Underlying conditions in the transfusion recipient seem important in TRALI, and the incidence of such reactions may be much higher in patients with hypoxia and/or underlying endothelial cell injury [7,8]. In light of extremely low serum IgG and IgG1 levels, it is likely that the abrupt infusion of exogenous IgG that remained in the transfused blood product may have been responsible for the potential exacerbation of an underlying risk, resulting in TLE.

ACKNOWLEDGMENTS

The authors thank Ms. Ramona M. Rodriguiz, B.S., Pediatric Endocrinology, for her efforts in obtaining patient specimens and records. The authors also thank Amy Hawn, Ph.D., and Ms. Debbie Richardson, M.T. (A.S.C.P.), C.H.S.., A.B.H.I., Tissue Typing Laboratory, for their excellent help in screening the sera for HLA antibodies. This work was supported in part by US Food and Drug Administration Orphan Drug Grant FD-R000768 (to L.L.K. and P.R.M.), by the Children's Hospital Fund of the Medical University of South Carolina (to L.L.K.), and by Transfusion Medicine Academic Award KO7HL02530-01A1, from the National Heart, Lung, and Blood Institute of the NIH (to E.K.J.).

REFERENCES

1. Key LL, Carnes D, Cole S: Treatment of congenital osteopetrosis with high-dose calcitriol. N Engl J Med 310:409–415, 1984.
2. Key LL, Rodriguiz RM, Willi SM: Long-term treatment of osteopetrosis with recombinant human interferon gamma. N Engl J Med 332:1594–1599, 1995.
3. Key LL, Rodriguiz RM, Wang WC: Cytokines and bone resorption in osteopetrosis. Int J Pediatr Hematol Oncol 2:143–149, 1995.
4. Madyastha PR, Madyastha KR, Wade T, Levine DH: An improved method for rapid layering of Ficoll-Hypaque double density gradients suitable for granulocyte separation. J Immunol Methods 48:281–284, 1982.
5. Madyastha PR, Glassman AB: Characterization of neutrophil agglutinins in primary autoimmune neutropenia of early childhood. Ann Clin Lab Sci 18:367–373, 1988.
6. Madyastha PR, Glassman AB: Detection of granulocyte antibodies by flow cytometry. Ann Clin Lab Sci 17:267–268, 1987.
7. Popovsky MA, Moore SB: Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. Transfusion 25:573–577, 1985.
8. Van Buren NL, Stroncek DF, Clay ME, McCullough J, Dalmasso AP: Transfusion-related acute lung injury caused by an NB2 granulocyte-specific antibody in a patient with thrombotic thrombocytopenic purpura. Transfusion 30:42–45, 1990.
9. Skolnick AA: Transfusion medicine faces time of major challenges and changes. JAMA 268:697, 1992.
10. Madyastha PR, Jeter EK, Key LL: Upregulation of the high affinity Fc receptor for IgG (FγRI;CD64) on granulocytes from patients with osteopetrosis: An intrinsic defect (abstract). Ann Clin Lab Sci 25:350, 1995.
11. Huizinga TWJ, van der Schoot CE, Weening RS: Induction of neutrophil Fc receptor I expression can be used as a marker for biologic activity of recombinant interferon-γ in vivo. Blood 77:2088–2090, 1991.
12. Repp R, Valerius T, Sendler A: Neutrophils express the high affinity receptor for IgG (FcγRI, CD64) after in vivo application of recombinant human granulocyte colony-stimulating factor. Blood 78:885–889, 1991.
13. Verhoef G, Boogaerts M: Treatment with granulocyte-macrophage colony stimulating factor and the adult respiratory distress syndrome. Am J Hematol 36:285–287, 1991.
14. Boogaerts MA, Demuynck H, Schetz M, Zachee P, Verhoef G: ARDS during G-CSF therapy for drug-induced agranulocytosis. Blood [Suppl]82:495, 1993.
15. Buckle AM, Hogg N: The effect of IFN-γ and colony-stimulating factors on the expression of neutrophil cell membrane receptors. J Immunol 43:2295–2301, 1989.
16. Berger M, Wetzler EM, Wallis RS: Tumor necrosis factor is the major monocyte product that increases complement receptor expression on mature human neutrophils. Blood 71:151–158, 1988.
17. Mantovani A, Dejana E: Cytokines as communication signals between leukocytes and endothelial cells. Immunol Today 10:370–375, 1989.